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FORM PTO-1390 (Modified)
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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

BB1095

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

INTERNATIONAL APPLICATION NO.
PCT/US998/13992INTERNATIONAL FILING DATE
7 JULY 1998 (07.07.98)PRIORITY DATE CLAIMED
11 JULY 1997 (11.07.97)TITLE OF INVENTION
PLANT SU1 HOMOLOGSAPPLICANT(S) FOR DO/EO/US
ODELL, Joan Tellefsen, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☒ A change of power of attorney and/or address letter.
18. ☒ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

17. General Power of Attorney
18. Express Mail Label No.: EJ236616789US
19. Petition to Revoke

U.S. APPLICATION NO. (IF KNOWN, SERIAL NO.)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
09/462972		PCT/US98/13992		BB1095	
20. The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :					
<input checked="" type="checkbox"/> Search Report has been prepared by the EPO or JPO				\$840.00	
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482)				\$670.00	
<input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))				\$760.00	
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO				\$970.00	
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)				\$96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$840.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$0.00	
CLAIMS		NUMBER FILED		NUMBER EXTRA	
Total claims		10 - 20 =		0	
Independent claims		4 - 3 =		1	
Multiple Dependent Claims (check if applicable).					
TOTAL OF ABOVE CALCULATIONS =				\$918.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).				\$0.00	
SUBTOTAL =				\$918.00	
Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$918.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				\$40.00	
TOTAL FEES ENCLOSED =				\$958.00	
				Amount to be refunded	
				charged	
<input type="checkbox"/> A check in the amount of to cover the above fees is enclosed.					
<input checked="" type="checkbox"/> Please charge my Deposit Account No. 04-1928 in the amount of \$958.00 to cover the above fees. A duplicate copy of this sheet is enclosed.					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 04-1928 A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
FEULNER, Gregory J. E.I. DU PONT DE NEMOURS AND COMPANY LEGAL PATENT RECORDS CENTER 1007 MARKET STREET WILMINGTON, DELAWARE 19898 US			SIGNATURE Bart E. Lerman Reg. No. 31,897 for FUELNER, Gregory J. NAME 41,744 REGISTRATION NUMBER 14 January 2000 DATE		

TITLE

PLANT SUG1 HOMOLOGS

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding coactivator proteins involved in regulation of gene expression in plants and seeds. These same proteins also function as regulatory components of the 26S proteasome which is involved in regulation of protein turnover.

BACKGROUND OF THE INVENTION

Gene expression levels are influenced by the interactions of transcription factors with proteins that are present in general transcription complexes. In addition, other non-DNA binding proteins known as coactivators interact with transcription factors and transcription complex proteins to further stimulate transcription. These coactivators are thought to be mediators of the transcription response. One type of coactivator is represented by the SUG1 gene, which has been identified in several types of eucaryotic cells such as mouse, yeast and *Xenopus* cells. SUG1 proteins have a coiled coil domain and an "AAA" domain (i.e., ATPases associated with a variety of cellular activities; see vom Bauer et al. (1996) *EMBO J.* 15:110-124).

SUG1 proteins have been shown to bind to the activation domains of the transcription factors, for example yeast SUG1 has been shown to bind to GAL4 of yeast and VP16 of herpesvirus (Swaffield et al. (1995) *Nature* 374:88-91). A mutant GAL4 that lacks the activation domain and therefore cannot activate transcription recovers this function in the presence of a mutant yeast SUG1, indicating a coactivation role for SUG1 (Swaffield et al. (1992) *Nature* 357:698-700). Yeast SUG1 has also been shown to associate with the TATA-binding protein of the TFIID transcription complex *in vivo* and *in vitro*, indicating an intermediary role in gene activation (Swaffield et al. (1995) *Nature* 374:88-91).

Mouse SUG1 has been shown to be able to complement a yeast SUG1 mutant, suggesting that the mouse SUG1 also functions as a transcription coactivator (vom Bauer et al. *supra*). The human thyroid receptor interacting protein 1 (Trip1), which differs from the mouse SUG1 protein by only three amino acids, also complements a yeast SUG1 mutant and binds to the Gal4 and VP16 activation domains (Lee et al. (1995) *Nature* 374:91-94). Interestingly, Trip1 also binds to the thyroid-hormone receptor and the retinoid-X receptor in ligand-dependent fashion which further suggests that Trip1 plays a role in mediating transcription.

The mouse SUG1 protein may also play a role in protein degradation. Amino acid comparisons indicate that the mouse SUG1 sequence differs by only a single amino acid residue from the human p45 protein, which was isolated as a subunit of the PA700 proteasome regulatory complex (vom Bauer et al. (1996) *EMBO J* 15:110-124). PA700 includes a set of regulatory proteins that are associated with the 26S proteasome, a complex

that carries out selective degradation of abnormal proteins and naturally short-lived proteins related to cell cycle control and metabolic regulation (Akiyama et al. (1995) *FEBS Letters* 363:151-156).

To date no SUG1 protein homologs have been reported in plants. Because these proteins are involved in transcriptional regulation they are obvious targets for manipulating gene regulation in eukaryotes and as possible targets for screening assays for crop protection chemicals. There is a great deal of interest in identifying the genes that encode SUG1 homologs in plants. These genes may be used to express SUG1 proteins in plant cells to manipulate gene expression. Accordingly, the availability of nucleic acid sequences encoding all or a portion of a SUG1 protein would facilitate studies to better understand and alter gene expression in plants.

SUMMARY OF THE INVENTION

The instant invention relates to an isolated nucleic acid fragment encoding a plant coactivator involved in regulation of gene expression. Moreover, the protein encoded by the isolated nucleic acid fragment also regulates protein turnover. More particularly, this invention concerns isolated nucleic acid fragments encoding plant (specifically from maize, soybean, rice and wheat) homologs of the mouse SUG1 transcriptional coactivator. In addition, this invention relates to a nucleic acid fragment that is complementary to a nucleic acid fragment encoding a plant SUG1 protein.

In another embodiment, the instant invention relates to a chimeric gene that comprises a nucleic acid fragment encoding a plant SUG1 protein, or to a chimeric gene that comprises a nucleic acid fragment that is complementary to a nucleic acid fragment encoding a plant SUG1 protein, the nucleic acid fragment operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in production of levels of the encoded protein in transformed host cells that are altered (i.e., increased or decreased) relative to the levels produced in untransformed host cells.

In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene comprising a nucleic acid fragment encoding a plant SUG1 protein or a chimeric gene comprising a nucleic acid fragment that is complementary to the nucleic acid fragment encoding a plant plant SUG1 protein, the chimeric gene operably linked to suitable regulatory sequences. Expression of the chimeric gene results in production of altered levels of protein encoded by the operably linked nucleic acid fragment in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

An additional embodiment of the instant invention concerns a method of altering the level of expression of a plant SUG1 protein in a transformed host cell comprising:

a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding

a plant SUG1 protein or a chimeric gene that comprises a nucleic acid fragment that is complementary to the nucleic acid fragment encoding a plant SUG1 protein; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of the protein encoded by the operably linked nucleic acid fragment in the transformed host cell.

An addition embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or substantially all of an amino acid sequence encoding a plant SUG1 protein.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of a plant SUG1 protein, the method comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant SUG1 protein operably linked to suitable regulatory sequences; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the plant SUG1 protein in the transformed host cell; (c) optionally purifying the plant SUG1 protein expressed by the transformed host cell; (d) treating the plant SUG1 protein with a compound to be tested; and (e) comparing the activity of the plant SUG1 protein that has been treated with a test compound to the activity of an untreated plant SUG1 protein, thereby selecting compounds with potential for inhibitory activity.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawings and sequence descriptions which form a part of this application.

Figure 1 shows a comparison of the amino acid sequences of the mouse SUG1 protein (Z54219), the human p45 protein (P47210), the human Trip1 transcriptional coactivator (L38810), the *Saccharomyces cerevisiae* SUG1 transcriptional coactivator (X66400), and the instant soybean and maize SUG1 protein homologs (se1.pk0023.b5 and cs1.pk0051.b7, respectively).

Figure 2 shows a comparison of the amino acid sequences of the mouse SUG1 protein SEQ ID NO:7 (3), the instant soybean clone se1.pk0023.b5 (2), a corn contig (cs1.pk0051.b7, cr1n.pk0096.b2 and cta1n.pk0056.c11) (1) and a wheat clone (wl1n.pk0053.g3) (4).

The following sequence descriptions and sequence listings attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

SEQ ID NO:1 is the nucleotide sequence comprising the entire cDNA insert in clone se1.pk0023.b5 encoding a soybean SUG1 protein.

SEQ ID NO:2 is the deduced amino acid sequence of a soybean SUG1 homolog derived from the nucleotide sequence of SEQ ID NO:1.

SEQ ID NO:3 is the nucleotide sequence comprising the entire cDNA insert in clone cs1.pk0051.b7 encoding corn SUG1 protein.

5 SEQ ID NO:4 is the deduced amino acid sequence of a portion of a maize SUG1 homolog derived from the nucleotide sequence of SEQ ID NO:3.

SEQ ID NO:5 is the nucleotide sequence comprising a contig assembled from the cDNA inserts in clones cs1.pk0051.b7, cr1n.pk0096.b2 and cta1n.pk0056.c11 encoding a corn SUG1 protein.

10 SEQ ID NO:6 is the deduced amino acid sequence of a SUG1 protein derived from the nucleotide sequence of SEQ ID NO:5.

SEQ ID NO:7 is the amino acid sequence encoding the mouse SUG1 protein having EMBL accession No. Z54219.

15 SEQ ID NO:8 is the amino acid sequence encoding the *Saccharomyces cerevisiae* SUG1 transcriptional coactivator having EMBL accession No. X66400.

SEQ ID NO:9 is the amino acid sequence encoding the human Trip1 transcriptional coactivator having GenBank accession No. L38810.

SEQ ID NO:10 is the amino acid sequence encoding the human p45 protein having SWISS-PROT accession No. P47210.

20 SEQ ID NO:11 is the nucleotide sequence comprising a portion of a cDNA insert in clone wl1n.pk0053.g3 encoding a wheat SUG1.

SEQ ID NO:12 is the deduced amino acid sequence of a wheat SUG1 protein from the nucleotide sequence of SEQ ID NO:11.

25 SEQ ID NO:13 is the nucleotide sequence comprising a portion of a cDNA insert in clone rlr6.pk0064.e10 encoding a rice 26S protease regulatory subunit 8.

SEQ ID NO:14 is the deduced amino acid sequence of a 26S protease regulatory subunit 8 protein from the nucleotide sequence of SEQ ID NO:13.

30 The Sequence Descriptions contain the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IYUB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

35 The amino acid sequence similarity between the instant plant SUG1 proteins and the mouse and yeast SUG1 proteins, human Trip1 and p45 proteins indicates that the plant SUG1 proteins may function as transcriptional coactivators and as regulators of protein degradation (Figure 1). The plant proteins may therefore be used to reduce expression of specific genes whose promoters are normally regulated by SUG1, using antisense or

co-suppression technology. The instant plant proteins may also be used to enhance gene expression of those genes whose promoters are normally targeted by the transcription factors that the plant SUG1 proteins normally interact with.

Alternatively, the plant SUG1 protein coactivation function can be targeted to a novel promoter region by the addition of either a DNA binding domain or a protein-protein interaction domain. The instant plant SUG1 protein can be fused to a very defined DNA-binding domain, such as, but not limited to, a bacterial lexA DNA binding domain, a yeast Gal4 DNA-binding domain or a DNA binding domain from a plant transcription factor. On the other hand, a synthetic promoter can be designed to contain multiple copies of a target site which is necessary for the specific binding by either the lexA, Gal4 or plant DNA binding domain. By using this approach, the plant SUG1 protein can be specifically targeted to the engineered synthetic promoter, thus leading to a higher level of gene expression when used in combination with an interacting transcription factor.

Additionally, plant SUG1 proteins can be fused to a transcription factor that already includes its own DNA binding domain in order to target the coactivator. Besides DNA-binding domains, the plant SUG1 proteins can also be fused to other transcription regulatory proteins, such as transcription mediators. Normally, these mediators do not bind to DNA directly and are recruited to their target sites by interaction with other DNA-binding proteins. By fusing the plant SUG1 protein to these mediators, the plant SUG1 protein can be targeted to specific regulatory elements through the interaction between the mediators and other DNA-binding proteins.

In the context of this disclosure, a number of terms shall be utilized. As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA. As used herein, "contig" refers to an assemblage of overlapping nucleic acid sequences to form one contiguous nucleotide sequence. For example, several DNA sequences can be compared and aligned to identify common or overlapping regions. The individual sequences can then be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate alteration of gene

expression by antisense or co-suppression-technology or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary sequences.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a gene which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded protein, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Moreover, the skilled artisan recognizes that substantially similar nucleic acid sequences encompassed by this invention are also defined by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C), with the sequences exemplified herein. Preferred substantially similar nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are 85% identical to the coding sequence of the nucleic acid fragments reported herein. More preferred nucleic acid fragments are 90% identical to the coding sequence of the nucleic acid fragments reported herein. Most preferred are nucleic acid fragments that are 95% identical to the coding sequence of the nucleic acid fragments reported herein. The percent identity used herein, can be precisely determined by the DNASTAR protein alignment protocol using the Hein algorithm (Hein, J.J. (1990) Unified Approach to Alignment and Phylogenies. *Methods in Enzymology*, vol. 183, 626-645). Default parameters for the J. J. Hein method for multiple alignments are: GAP PENALTY=11, GAP LENGTH PENALTY=3; for pairwise alignments KTUPLE 6.

A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to afford putative identification of that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide

or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches partial or complete amino acid and nucleotide sequences encoding a particular plant protein. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding a corn, soybean, rice or wheat SUG1 protein as set forth in SEQ ID NOs:2, 4, 6, 12 and 14. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic genes" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments which are then enzymatically assembled to construct the entire gene. "Chemically synthesized", as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of DNA may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that encodes a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its

own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg, (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G.D. (1995) *Molecular Biotechnology* 3:225).

The "3' non-coding sequences" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al., (1989) *Plant Cell* 1:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript. Alternatively, the RNA transcript may be an RNA sequence derived from posttranscriptional processing of the primary transcript; this is referred to as the mature RNA. "Messenger RNA" (mRNA) refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to an RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Pat. No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Pat. No. 5,231,020).

"Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

“Transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. Examples of methods of plant transformation include Agrobacterium-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or “gene gun” transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Pat. No. 4,945,050).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter “Maniatis”).

This invention relates to plant cDNAs with homology to the mouse, yeast and *Xenopus* SUG1 proteins. The cDNAs have been isolated and identified by comparison of random plant cDNA sequences to several publically available databases using the BLAST algorithms well known to those skilled in the art. The nucleotide sequence of a soybean cDNA clone (se1.pk0023.b5) encoding a SUG1 homolog is provided in SEQ ID NO:1, and the deduced amino acid sequence is provided in SEQ ID NO:2. The nucleotide sequence of a maize SUG1 homolog (cs1.pk0051.b7) is provided in SEQ ID NOS:3 and 5, and the deduced amino acid sequence is provided in SEQ ID NO:4 and 6. The nucleotide sequence of a wheat SUG1 homolog (wl1n.pk0053.g3) is provided in SEQ ID NO:11, and the deduced amino acid sequence is provided in SEQ ID NO:12. Lastly, the nucleotide sequence of a rice 26S protease regulatory subunit 8 homolog (rlr6.pk0064.e10) is provided in SEQ ID NO:13, and the deduced amino acid sequence is provided in SEQ ID NO:14. Homologs of these proteins from other plants can now be identified by comparison of random cDNA sequences to the maize, soybean, and wheat sequences provided herein.

The full insert of cDNA clone se1.pk0023.b5 encoding the soybean SUG1 homolog has been completely sequenced. Amino acid sequence comparison indicates that there is 80% sequence identity between this soybean homolog and the mouse SUG1 protein (Figure 1). Sequence alignments and percent identity calculations were performed by the Jotun Hein method using the Megalign program of DNASTar™ sequence analysis software (DNASTAR Inc. 1228 South Park Street, Madison Wisconsin, 53715)

Based on the high sequence homology, it is believed that this soybean clone encodes a plant homolog of mouse SUG1. This is the first indication that there is a similar SUG-mediated gene activation system in plants.

Likewise, the full insert of cDNA clone cs1.pk0051.b7 encoding the maize SUG1 homolog has been completely sequenced. The region encoding approximately thirty amino acids is missing from the 5' end of the cDNA insert. At the amino acid level, this maize peptide is approximately 95% identical to the soybean SUG1 protein encoded by cDNA clone se1.pk0023.b5. Nucleotide identity between the soybean and maize cDNAs is approximately 78%. Sequence alignments and percent identity calculations were performed

by the Jotun Hein method using the Megalign program of DNASTar™ sequence analysis software (DNASTAR Inc. 1228 South Park Street, Madison Wisconsin, 53715).

The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding other homologs of SUG1 from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other plant homologs of SUG1 either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part of or full-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency. Genomic fragments can be isolated that include the promoter region that directs expression of the plant SUG1 protein. This promoter may be prepared as a DNA fragment including regulatory elements with or without the untranslated leader and used in expression of other coding regions or for co-suppression.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al., (1988) *PNAS USA* 85:8998) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al., (1989) *PNAS USA* 86:5673; Loh et al., (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be

combined to generate full-length cDNAs (Frohman, M.A. and Martin, G.R., (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner, R.A. (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed plant homologs of SUG1 are present at higher or lower levels than normal or in cell types or developmental stages in which it is not normally found. This would have the effect of altering the level of a plant SUG1 protein in those cells.

Overexpression of plant a SUG1 protein may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise a promoter sequence and translation leader sequence derived from the same gene. A 3' non-coding sequence encoding a transcription termination signal may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) *EMBO J.* 4:2411-2418; De Almeida et al., (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications, it may be desirable to reduce or eliminate expression of the genes encoding the instant plant of SUG1 homologs. In order to accomplish this, chimeric genes designed for co-suppression of the instant plant SUG1 protein genes can be constructed by linking the genes or gene fragments encoding the plant SUG1 proteins to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragments in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via

transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

5 The instant plant SUG1 homologs (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the SUG1 proteins by methods well known to those skilled in the art. The antibodies are useful for detecting plant SUG1 proteins *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant SUG1 proteins are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those
10 skilled in the art. Any of these could be used to construct chimeric genes for production of the instant plant SUG1 homologs. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of corn, soybean or wheat SUG1 proteins. An example of a vector for high level expression of the instant plant SUG1 homologs in a bacterial host is provided (Example 6).

15 Additionally, the instant SUG1 proteins can be used as targets to facilitate design and/or identification of inhibitors of the activity of the protein that may be useful as herbicides. This is desirable because the protein described plays a key role in regulation of gene expression. Accordingly, inhibition of the activity of the proteins described herein could lead to inhibition of gene expression sufficient to inhibit plant growth. Thus, the
20 instant SUG1 proteins could be appropriate for new herbicide discovery and design.

All or a portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to expression of the instant plant SUG1 proteins. Such information may be useful in plant breeding in order to develop lines with desired phenotypes.

25 For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al., (1987) *Genomics* 1:174-181) in order to
30 construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population
35 (Botstein, D. et al., (1980) *Am.J.Hum.Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in R. Bernatzky, R. and Tanksley, S.D. (1986) *Plant Mol.Biol.Reporter* 4(1):37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross

populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

5 Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel, J. D., et al., In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

10 In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask, B. J. (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan, M. et al. (1995) *Genome Research* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

15 A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian, H. H. (1989) *J. Lab. Clin. Med.* 114(2):95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield, V. C. et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren, U. et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov, B. P. (1990) *Nucleic Acid Res.* 18:3671), Radiation
20 Hybrid Mapping (Walter, M. A. et al. (1997) *Nature Genetics* 7:22-28) and Happy Mapping (Dear, P. H. and Cook, P. R. (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping,
25 it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

30 Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer, (1989) *Proc. Natl. Acad. Sci USA* 86:9402; Koes et al., (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al., (1995) *Plant Cell* 7:75). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence
35 primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant SUG1 gene. Alternatively, the SUG1 gene may be used as a hybridization probe against PCR amplification products generated from the

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mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous SUG1 gene can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the plant SUG1 gene product.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from soybean embryo obtained seven days after flowering and maize leaf sheath obtained from five week old *Zea mays* B73 maize plants were prepared. cDNA libraries representing mRNAs from wheat leaf from 7 day old etiolated seedlings (normalized, normalized essentially as described in U.S. Pat. No. 5,482,845) were prepared. A cDNA library representing mRNAs from rice leaf 15 days after germination and 6 hours after infection of strain *Magaporthe grisea* 4360-R-62 (AVR2-YAMO) was also prepared.

Separate cDNA libraries representing the maize, soybean, rice and wheat cDNAs were prepared in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). Conversion of the Uni-ZAP™ XR libraries into plasmid libraries was accomplished according to the protocol provided by Stratagene. Upon conversion, cDNA inserts were contained in the plasmid vector pBluescript. cDNA inserts from randomly picked bacterial colonies containing recombinant pBluescript plasmids were amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs were sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams, M. D. et al., (1991) *Science* 252:1651). The resulting ESTs were analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification and Characterization of cDNA Clones

ESTs encoding homologs of the mouse, yeast and *Xenopus* SUG1 proteins were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-

redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding SUG1 Homologs

The BLASTX search using the EST sequences from clones se1.pk0023.b5, cs1.pk0051.b7, cr1n.pk0096.b2 and ct1n.pk0056.c11 revealed similarity of the proteins encoded by the cDNAs to the SUG1 protein from mouse (EMBL Accession No. Z54219). The BLASTX search using the EST sequence from clone w11n.pk0053.g3 revealed similarity of the protein encoded by the cDNA to the SUG1 protein from *Xenopus laevis* (GenBank Accession No. X81986). The BLASTX search using the EST sequence from clone rlr6.pk0064.e10 revealed similarity of the protein encoded by the cDNA to the 26S Protease Regulatory Subunit 8 (a SUG1 protein homolog) from *Dictyostelium discoideum* (SWISS PROT Accession No. P34124). The BLAST results for each of these ESTs are shown in Table 3:

TABLE 1
BLAST Results for Clones Encoding Polypeptides Homologous to SUG1 Proteins

Clone	BLAST pLog Score (Organism Accession Number)	
se1.pk0023.b5	35.05	(Z54219)
cs1.pk0051.b7	27.85	(Z54219)
Contig composed of:	217.03	(Z54219)
cs1.pk0051.b7		
ta1n.pk0056.c11		
cr1n.pk0096.b2		
w11n.pk0053.g3	21.62	(X81986)
rlr6.pk0064.e10	53.19	(P34124)

In the process of comparing the corn ESTs it was found that clones cs1.pk0051.b7, cr1n.pk0096.b2 and cta1n.pk0056.c11 had overlapping regions of homology. Using this homology it was possible to align the entire cDNA insert of clone cs1.pk0051.b7, with the ESTs of clones cr1n.pk0096.b2 and cta1n.pk0056.c11 and assemble a contig (a contig is an assemblage of overlapping nucleic acid sequences to form one contiguous nucleotide sequence). The individual sequences were assembled into a unique contiguous nucleotide sequence encoding a unique corn SUG1 protein. The nucleotide sequence of the contig is shown in SEQ ID NO:5; the deduced amino acid sequence of the contig is shown in SEQ ID NO:6. The nucleotide sequence of the cDNA clone w11n.pk0053.g3 is shown in SEQ ID NO:11; the deduced amino acid sequence encoded by clone w11n.pk0053.g3 is shown in SEQ ID NO:12. The nucleotide sequence of the cDNA clone rlr6.pk0064.e10 is shown in SEQ ID NO:13; the deduced amino acid sequence encoded by clone rlr6.pk0064.e10 is shown in SEQ ID NO:14. BLAST scores and probabilities indicate that the instant nucleic acid fragments encode portions of a plant SUG1 protein. These sequences represent plant sequences encoding a SUG1 protein.

The sequence of the entire cDNA insert in clone se1.pk0023.b5 was determined and is shown in SEQ ID NO:1; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:2. The amino acid sequence set forth in SEQ ID NO:2 was evaluated by BLASTP, yielding a pLog value of 210.70 versus the mouse SUG1 sequence. The sequence of the entire maize contig containing clones cs1.pk0051.b7, cta1n.pk0056.c11 and cr1n.pk0096.b2 was determined and is shown in SEQ ID NO:5; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:6. The amino acid sequence set forth in SEQ ID NO:6 was evaluated by BLASTP, yielding a pLog value of 217.03 versus the mouse SUG1 sequence. Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:2 the mouse SUG1 sequence. Figure 2 presents an alignment of the amino acid sequence set forth in SEQ ID NO:6 and the mouse SUG1 protein. The data in Table 2 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2 and 6 and the mouse SUG1 sequence.

TABLE 2
Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to SUG1

Clone	SEQ ID NO.	Percent Identity to Z54219
se1.pk0023.b5	2	80
Contig composed of:	6	81
cs1.pk0051.b7		
cta1n.pk0056.c11		
cr1n.pk0096.b2		

Sequence alignments and percent identity calculations were performed by the Jotun Hein method using the Megalign program of DNASTar™ sequence analysis software (DNASTAR Inc. 1228 South Park Street, Madison Wisconsin, 53715).

- 5 Sequence alignments and BLAST scores and probabilities indicate that the instant nucleic acid fragments encode entire or nearly entire SUG1 protein. These sequences represent the first plant sequences encoding a SUG1 protein.

EXAMPLE 4

Expression of Chimeric Genes in Monocot Cells

- 10 A chimeric gene comprising a cDNA encoding a plant SUG1 homolog in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of
- 15 the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest
- 20 Treaty at ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight,
- 25 essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene
- 30 encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding a plant SUG1 protein, and the 10 kD zein 3' region.

- The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated
- 35 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum

of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al., (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a KaptonTM flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a BiolisticTM PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al., (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant plant SUG1 protein in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

A nucleic acid fragment encoding a plant SUG1 protein may be generated by polymerase chain reaction (PCR) of the instant cDNA clones using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding a plant SUG1 protein. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Kline et al. (1987) *Nature* (London) 327:70, U.S. Patent No. 4,945,050). A Du Pont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et

al.(1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the plant

5 SUG1 protein, and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 μ L of a 60 mg/mL 1 μ m gold particle suspension is added (in order): 5 μ L DNA (1 μ g/ μ L), 20 μ L spermidine (0.1 M), and 50 μ L CaCl_2 (2.5 M). The particle preparation is

10 then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μ L 70% ethanol and resuspended in 40 μ L of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μ L of the DNA-coated gold particles are then loaded on each macro carrier disk.

15 Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the

20 retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post

25 bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or

30 regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 6

Expression of Chimeric Genes in Microbial Cells

For expression of a plant SUG1 protein, in a microbial cell, a cDNA encoding the instant plant SUG1 protein can be inserted into the T7 *E. coli* expression vector pBT430.

35 This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for

insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

5 Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the SUG1 protein coding sequence. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the plant SUG1 protein are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 7

Evaluating Compounds for Their Ability to Inhibit Plant SUG1 Protein Activity

The plant SUG1 homologs described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 5, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The

instant polypeptide may be expressed either as a mature form of the protein as observed *in vivo* or as a fusion protein by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("His₆"). The fusion protein may be engineered with a protease recognition site at the fusion point so that fusion partner can be separated by protease digestion to yield an intact, mature polypeptide. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the plant SUG1 polypeptide.

Purification of the instant polypeptide, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the polypeptide is expressed as a fusion protein, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed polypeptide or an affinity resin containing ligands which are specific for the polypeptide. For example, polypeptide may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a (His)₆ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β-mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the plant SUG1 protein may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified polypeptide, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit the activity of a plant SUG1 protein. Assays may be conducted under well known experimental conditions which permit optimal activity. An example of an *in vitro* assay for transcriptional coactivation activity is described by vom Bauer et al. ((1996) *EMBO J.* 15:110-124). The skilled artisan is well aware of simple modifications that could be made to the published protocols that would afford detection of inhibitors the plant SUG1 protein.

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding a plant SUG1 protein comprising a member selected from the group consisting of:

- 5 (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs:2, 4, 6, 12, and 14;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs:2, 4, 6, 12, and 14; and
- 10 (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

2. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment is set forth in a member selected from the group consisting of SEQ ID NOs:1, 3, 5, 11, and 13.

15

3. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.

4. A transformed host cell comprising the chimeric gene of Claim 3.

5. A method of altering the level of expression of a plant SUG1 protein in a host cell comprising:

20

- (a) transforming a host cell with the chimeric gene of Claim 3; and
- (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of a plant SUG1 protein in the transformed host cell.

25

6. A method of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a plant SUG1 protein comprising:

- (a) probing a cDNA or genomic library with the nucleic acid fragment of Claim 1;
- 30 (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of Claim 1;
- (c) isolating the DNA clone identified in step (b); and
- (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

35 wherein the sequenced nucleic acid fragment encodes all or substantially all of the amino acid sequence encoding a plant SUG1 protein.

7. A method of obtaining a nucleic acid fragment encoding a portion of an amino acid sequence encoding a plant SUG1 protein comprising:

- 5 (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs:1, 3, 5, 11, and 13; and
(b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a portion of an amino acid sequence encoding a plant SUG1 protein.

10 8. The product of the method of Claim 6.

9. The product of the method of Claim 7.

10. A method for evaluating at least one compound for its ability to inhibit the activity of a plant SUG1 protein, the method comprising the steps of:

- 15 (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant SUG1 protein, operably linked to suitable regulatory sequences;
(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the SUG1 protein encoded by the operably
20 linked nucleic acid fragment in the transformed host cell;
(c) optionally purifying the SUG1 protein expressed by the transformed host cell;
(d) treating the SUG1 protein with a compound to be tested; and
25 (e) comparing the activity of the SUG1 protein that has been treated with a test compound to the activity of an untreated SUG1 protein,

thereby selecting compounds with potential for inhibitory activity.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US93/07369 (22) International Filing Date: 10 August 1993 (10.08.93) (30) Priority data: 9217381.4 14 August 1992 (14.08.92) GB (71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). (72) Inventor; and (75) Inventor/Applicant (for US only) : SPELTHANN, Heinz, H. [DE/CH]; 54, route du Creux-de-Boisset, CH-1286 Soral (CH). (74) Agents: EVANS, Craig, H. et al.; E.I. du Pont de Nemours and Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).		(81) Designated States: BR, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: FLEXIBLE POLAR THERMOPLASTIC POLYOLEFIN COMPOSITIONS (57) Abstract Polar thermoplastic polyolefin blends are provided which are flexible and are particularly useful in replacing polyvinyl chloride sheeting used as liners, folders, etc. The blends comprise, in general, a non-polar thermoplastic polyolefin, a polar ethylene copolymer, and an acid grafted olefin polymer as a compatibilizing agent, all of which are chlorine-free.		

254219 MALDCEQMELEEG-----KAGSGLROYVLSKIEELQILVANDKSONIRLQAORNELNAKVRLLREELQILOEQSSVGEVWRAMDKKVLVKV 89
 P47210 MALDCEQMELEEG-----KAGSGLROYVLSKIEELQILVANDKSONIRLQAORNELNAKVRLLREELQILOEQSSVGEVWRAMDKKVLVKV 89
 L38810 MALDCEQMELEEG-----KAGSGLROYVLSKIEELQILVANDKSONIRLQAORNELNAKVRLLREELQILOEQSSVGEVWRAMDKKVLVKV 89
 X66400 MPAVTSSNIVLET-----HESGKIPYFEQKIQETELKIRSKTENGRRLEPAQRNALNDKVRITKOEIRLLOEPGSSVGEVHGVSKKVLVKV--88
 sel.pk0023.b5 MALVGVELKHAAGVPEANCSPKTKQCEGLRHVYSINIEHQILLPKQTHNIARLEPAQRNDLNSRVMLREELQILOEQSSVGEVWAMCKKVLVKV 100
 cs1.pk0051.b7 E-----HIHDLQILQIRQKTHNIARLEPAQRNDLNSRVMLREELQILOEQSSVGEVWAMCKKVLVKV 64

254219 HPEGKFWVDKNIIDINDVTPNCRVAIRNDSTYTHKILPNKVDPLVSLMMWEKVPDSTYEMIGGLDKQIKEIKEVIELPVKHPELFEALGIAQPKGVLLY 189
 P47210 HPEGKFWVDKNIIDINDVTPNCRVAIRNDSTYTHKILPNKVDPLVSLMMWEKVPDSTYEMIGGLDKQIKEIKEVIELPVKHPELFEALGIAQPKGVLLY 189
 L38810 HPEGKFWVDKNIIDINDVTPNCRVAIRNDSTYTHKILPNKVDPLVSLMMWEKVPDSTYEMIGGLDKQIKEIKEVIELPVKHPELFEALGIAQPKGVLLY 189
 X66400 QPEGKYIVVAKOINVKDLKASQFVCLPSDSYMLHKVLENKADPLVSLMMWEKVPDSTYDMWGLTKQIKEIKEVIELPVKHPELFESIGIAQPKGVLLY 188
 sel.pk0023.b5 HPEGKYVVDIDKNIDITKITPSTRVAIRNDSTYVHLVLPKSVDPPLVNLMMWEKVPDSTYDMIGGLDQIQIKEIKEVIELPIKHPELFESIGIAQPKGVLLY 200
 cs1.pk0051.b7 HPEGKYVVDIDKSIDITKITPSTRVAIRNDSTYMLHLPLPSKVDPLVNLMMWEKVPDSTYDMIGGLDQIQIKEIKEVIELPIKHPELFESIGIAQPKGVLLY 164

254219 CPPGTGKTLARAVAHHTDCIFIRVSGSELVQKFTIGEGARWRELFVAREHAPSIIFMDEIDSIGSSRLEGSSG-GDSEVQRTMIELINQIDGFEATKN 288
 P47210 CPPGTGKTLARAVAHHTDCIFIRVSGSELVQKFTIGEGARWRELFVAREHAPSIIFMDEIDSIGSSRLEGSSG-GDSEVQRTMIELINQIDGFEATKN 288
 L38810 CPPGTGKTLARAVAHHTDCIFIRVSGSELVQKFTIGEGARWRELFVAREHAPSIIFMDEIDSIGSSRLEGSSG-GDSEVQRTMIELINQIDGFEATKN 288
 X66400 CPPGTGKTLARAVAHHTDCIFIRVSGSELVQKFTIGEGARWRELFVAREHAPSIIFMDEIDSIGSTRIVE-GSGGSDSEVQRTMIELINQIDGFEATKN 287
 sel.pk0023.b5 CPPGTGKTLARAVAHHTDCIFIRVSGSELVQKFTIGEGARWRELFVAREHAPSIIFMDEIDSIGSARWESGNGDSEVQRTMIELINQIDGFEASNK 300
 cs1.pk0051.b7 CPPGTGKTLARAVAHHTDCIFIRVSGSELVQKFTIGEGARWRELFVAREHAPSIIFMDEIDSIGSARWESGNGDSEVQRTMIELINQIDGFEASNK 264

FIG.1

09/462962

310 320 330 340 350 360 370 380 390 400
 IKVTATNRIDIDLSALLPGRIDRDKIEFFPPNEEARLDILKTHSRKANLTRGINLRKTAELMFGASGAEVKGVCTEAGMVALPERRVHVTOEDFEVAVA 388
 IKVTATNRIDIDLSALLPGRIDRDKIEFFPPNEEARLDILKTHSRKANLTRGINLRKTAELMFGASGAEVKGVCTEAGMVALPERRVHVTOEDFEVAVA 388
 IKVTATNRIDIDLSALLPGRIDRDKIEFFPPNEEARLDILKTHSRKANLTRGINLRKTAELMFGASGAEVKGVCTEAGMVALPERRVHVTOEDFEVAVA 388
 IKVTATNRIDIDLSALLPGRIDRDKIEFFPPNEEARLDILKTHSRKANLTRGINLRKTAELMFGASGAEVKGVCTEAGMVALPERRVHVTOEDFEVAVA 387
 IKVTATNRIDIDLSALLPGRIDRDKIEFFPPNEEARLDILKTHSRKANLTRGINLRKTAELMFGASGAEVKGVCTEAGMVALPERRVHVTOEDFEVAVA 400
 IKVTATNRIDIDLSALLPGRIDRDKIEFFPPNEEARLDILKTHSRKANLTRGINLRKTAELMFGASGAEVKGVCTEAGMVALPERRVHVTOEDFEVAVA 364
 IKVTATNRIDIDLSALLPGRIDRDKIEFFPPNEEARLDILKTHSRKANLTRGINLRKTAELMFGASGAEVKGVCTEAGMVALPERRVHVTOEDFEVAVA 364

254219

P47210

L38810

X66400

se1.pk0023.b5

cs1.pk0051.b7

410
 KVMKQSEKNSIKKLUK
 KVMKQSEKNSIKKLUK
 KVMKQSEKNSIKKLUK
 KVMKQSEKNSIKKLUK
 KVMKQSEKNSIKKLUK
 KVMKQSEKNSIKKLUK
 KVMKQSEKNSIKKLUK

254219

P47210

L38810

X66400

se1.pk0023.b5

cs1.pk0051.b7

406
 406
 406
 405
 418
 382

FIG.1 (CONTINUED)

FIGURE 2

1 MATVAMDISKPTPAASGDEAAAAAKGRSGGGEGGLRQYYLQHIHDLQLQIRQKTHNLNRL
2 MALVGVELKHAAEGVPEANCSA....KPTKQGEGLRHYYSLNIHEHQLLLRQKTHNLNRL
3 MALDGPEQMELEEG.....KAGSGLRQYYLSKIEELQLIVNDKSQNLRL
4 MATVAMDISKPTPVASGDEAAAAAKGRSGGGEGGLRQYYLQHIHDLQLQIRHKTTHNLNRL

1 EAQRNDLNSRVRMLREELQLLQEPGSYVGEVVKVMGKSKVLVKVHPEGKYVVDIDKSIDI
2 EAQRNDLNSRVRMLREELQLLQEPGSYVGEVVKVMGKNKVLVKVHPEGKYVVDIDKNIDI
3 QAQRNELNAKVRLLREELQLLQEQGSYVGEVVRAMDKKKVLVKVHPEGKFVVDVDKNIDI
4 EAQRNDLNSRVRMLREDXXLLXEPGSYVGXVVKAWGNQRFVVKVNPEGKXXVDIN.....

1 TKITPSTRVALRND SYMLHLILPSKVDPLVNL MKVEKVPDSTYDMIGGLDQOIKEIKEVI
2 TKITPSTRVALRND SYVLHLVLP SKVDPLVNL MKVEKVPDSTYDMIGGLDQOIKEIKEVI
3 NDVTPNCRVALRND SYTLHKILPNKVDPLVSLMMVEKVPDSTYEMIGGLDKOIKEIKEVI
4

1 ELPIKHPELFESLGIAQPKGVLLYGPPGTGKTLLARAVAHHTDCTFIRVSGSELVQKYIG
2 ELPIKHPELFESLGIAQPKGVLLYGPPGTGKTLLARAVAHHTDCTFIRVSGSELVQKYIG
3 ELPVKHPELFELGIAQPKGVLLYGPPGTGKTLLARAVAHHTDCTFIRVSGSELVQKFIG
4

1 EGSRMVRELFVMAREHAPSII FMDEIDSIGSARMESGTGNGDSEVQRTMLELLNQLDGFE
2 EGSRMVRELFVMAREHAPSII FMDEIDSIGSARMESGSGNGDSEVQRTMLELLNQLDGFE
3 EGARMVRELFVMAREHAPSII FMDEIDSIGSSRLEGGSG.GDSEVQRTMLELLNQLDGFE
4

1 ASNKIKVLMATNRIDILDQALLRPGRIDRKIEFPNPNEDSRFDILKIHSRKMNLMRGIDL
2 ASNKIKVLMATNRIDILDQALLRPGRIDRKIEFPTPNEESRLDILKIHSRRMNLMRGIDL
3 ATKNIKVIMATNRIDILDSALLRPGRIDRKIEFPNPNEEARLDILKIHSRKMNLTRGINL
4

1 KKIAEKMNGASGAELKAVCTEAGMFALRERRVHVTQEDFEMAVAKVMKKDTEKNMSLRKL
2 KKIAEKMNGASGAELKAVCTEAGMFALRERRVHVTQEDFEMAVAKVMKKETEKNNMSLRKL
3 RKIAELMPGASGAEVKGVCTEAGMYALRERRVHVTQEDFEMAVAKVMQKDSEKNMSIKKL
4

1 WK
2 WK
3 WK
4 ..

STP 58A1P0

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Rev. 06/95

0005 MAC A 1 07/10/1998 002

Docket Number
BB-1095-A

DECLARATION and POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PLANT SUG1 HOMOLOGS

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 7 July 1998 as U.S. Application No. _____ or PCT International Application No. PCT/US98/13992 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patent ability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Application No.	Country	Filing Date	Priority Claimed (Yes/No)
-----------------	---------	-------------	---------------------------

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States Provisional Application(s) listed below.

U.S. Provisional Application No.	U.S. Filing Date
----------------------------------	------------------

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application No.	Filing Date	Status (patented, pending or abandoned)
08/893,401	11 July 1997	Pending
PCT/US98/13992	07 July 1998	Pending

POWER OF ATTORNEY: I hereby appoint the following attorney(s) and/or agent(s) the power to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:Name: WILLIAM R. MAJARIANRegistration No.: 41,173

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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			Zip Code <u>19382</u>

☐ Additional Inventors are being named on separately numbered sheets attached hereto.

57P581100

09462972 011400

0005 HAL A1 07500911602 **GENERAL POWER OF ATTORNEY**
(Concerning Several International Patent Applications)

The undersigned, Howard J. Rudge, Senior Vice President and General Counsel of E. I. DU PONT DE NEMOURS AND COMPANY, 1007 Market Street, Wilmington, Delaware 19898 USA ("DuPont"), hereby confirms that the power to sign for DuPont has been granted to various individuals (as set forth in the attached excerpt from DuPont's Patent Board Rules of Procedure (January 1988), Appendix Section III.A.4), including the Chairman, Vice-Chairman, and those individuals who are Assistant Secretaries of the Patent Board. Currently these Assistant Secretaries are:

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Linda J. Davis	Dorothy W. Shafer
John E. Griffiths	Deborah A. Meginniss

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Fricke, Hilmar L.	<u>22,384</u>	Siege, Barbara C.	<u>30,684</u>
Golian, Andrew G.	<u>25,293</u>	Sinnott, Jessica M.	<u>34,015</u>
Gorman, Thomas W.	<u>31,959</u>	Steinberg, Thomas W.	<u>37,013</u>
Gould, David J.	<u>25,338</u>	Stevenson, Robert B.	<u>26,039</u>
Griffiths, John E.	<u>32,647</u>	Strickland, Frederick D.	<u>39,041</u>
Hamby, Jane O.	<u>32,872</u>	Tessari, Joseph A.	<u>32,177</u>
Hamby, William H.	<u>31,521</u>	Tulloch, Rebecca W.	<u>36,297</u>
Heiser, David E.	<u>31,366</u>	Walker, P. Michael	<u>32,602</u>
Hendrickson, John S.	<u>30,847</u>	Wang, Chen	<u>38,650</u>
Jones, Brian C.	<u>37,857</u>		

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The undersigned ratifies fully all actions already taken by the above-named individuals in accordance with the authority granted hereby.

E. I. DU PONT DE NEMOURS AND COMPANY

By: Howard J. Rudge
Howard J. Rudge
Senior Vice President and General Counsel

Date: 11/8/99

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
 - (A) ADDRESSEE: E. I. DUPONT DE NEMOURS AND COMPANY
 - (B) STREET: 1007 MARKET STREET
 - (C) CITY: WILMINGTON
 - (D) STATE: DELAWARE
 - (E) COUNTRY: UNITED STATES OF AMERICA
 - (F) ZIP: 19898
 - (G) TELEPHONE: 302-992-4926
 - (H) TELEFAX: 302-773-0164
 - (I) TELEX: 6717325
- (ii) TITLE OF INVENTION: PLANT SUG1 HOMOLOGS
- (iii) NUMBER OF SEQUENCES: 14
- (iv) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: DISKETTE, 3.50 INCH
 - (B) COMPUTER: IBM PC COMPATIBLE
 - (C) OPERATING SYSTEM: MICROSOFT WINDOWS 95
 - (D) SOFTWARE: MICROSOFT WORD VERSION 7.0A
- (v) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/893,401
 - (B) FILING DATE: JULY 11, 1997
- (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: MAJARIAN, WILLIAM R.
 - (B) REGISTRATION NUMBER: 41,173
 - (C) REFERENCE/DOCKET NUMBER: BB-1095-A

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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1254 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Soybean

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..1254

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG GCT CTT GTA GGA GTT GAA CTG AAG CAT GCG GCG GAG GGC GTA CCG	48
Met Ala Leu Val Gly Val Glu Leu Lys His Ala Ala Glu Gly Val Pro	
1 5 10 15	
GAG GCG AAT TGC TCC GCC AAG CCC ACC AAG CAG GGC GAG GGC CTC CGC	96
Glu Ala Asn Cys Ser Ala Lys Pro Thr Lys Gln Gly Glu Gly Leu Arg	
20 25 30	
CAC TAC TAT TCT CTC AAC ATC CAC GAG CAT CAG CTC CTT CTT CGC CAA	144
His Tyr Tyr Ser Leu Asn Ile His Glu His Gln Leu Leu Leu Arg Gln	
35 40 45	
AAG ACT CAT AAC CTC AAC CGT CTC GAG GCT CAG AGA AAC GAC CTC AAT	192
Lys Thr His Asn Leu Asn Arg Leu Glu Ala Gln Arg Asn Asp Leu Asn	
50 55 60	
TCT AGG GTG AGG ATG CTG CGC GAA GAA TTA CAG CTT CTG CAG GAA CCC	240
Ser Arg Val Arg Met Leu Arg Glu Glu Leu Gln Leu Leu Gln Glu Pro	
65 70 75 80	
GGC TCT TAT GTC GGT GAA GTT GTC AAA GTA ATG GGC AAG AAC AAA GTC	288
Gly Ser Tyr Val Gly Glu Val Val Lys Val Met Gly Lys Asn Lys Val	
85 90 95	
CTT GTC AAG GTC CAC CCA GAA GGA AAA TAT GTT GTT GAC ATT GAC AAA	336
Leu Val Lys Val His Pro Glu Gly Lys Tyr Val Val Asp Ile Asp Lys	
100 105 110	
AAT ATT GAC ATT ACA AAG ATT ACT CCA TCC ACT AGA GTT GCA CTC CGC	384
Asn Ile Asp Ile Thr Lys Ile Thr Pro Ser Thr Arg Val Ala Leu Arg	
115 120 125	
AAC GAC AGT TAT GTT CTT CAC TTA GTT CTG CCA AGT AAA GTT GAT CCA	432
Asn Asp Ser Tyr Val Leu His Leu Val Leu Pro Ser Lys Val Asp Pro	
130 135 140	
TTG GTC AAT CTG ATG AAA GTT GAG AAA GTT CCC GAT TCT ACA TAT GAC	480
Leu Val Asn Leu Met Lys Val Glu Lys Val Pro Asp Ser Thr Tyr Asp	
145 150 155 160	
ATG ATT GGT GGT TTA GAC CAG CAA ATT AAA GAA ATA AAA GAG GTC ATT	528
Met Ile Gly Gly Leu Asp Gln Gln Ile Lys Glu Ile Lys Glu Val Ile	
165 170 175	
GAG CTA CCA ATC AAA CAT CCT GAG CTG TTT GAA AGT CTT GGA ATT GCA	576
Glu Leu Pro Ile Lys His Pro Glu Leu Phe Glu Ser Leu Gly Ile Ala	
180 185 190	

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CAA CCA AAG GGT GTC CTG CTC TAT GGG CCA CCT GGT ACA GGT AAA ACA	624
Gln Pro Lys Gly Val Leu Leu Tyr Gly Pro Pro Gly Thr Gly Lys Thr	
195 200 205	
TTG TTG GCT AGG GCA GTG GCT CAT CAT ACT GAC TGT ACA TTC ATC AGG	672
Leu Leu Ala Arg Ala Val Ala His His Thr Asp Cys Thr Phe Ile Arg	
210 215 220	
GTG TCT GGT TCT GAG TTA GTT CAG AAA TAC ATT GGA GAA GGT TCT AGA	720
Val Ser Gly Ser Glu Leu Val Gln Lys Tyr Ile Gly Glu Gly Ser Arg	
225 230 235 240	
ATG GTC AGG GAA CTT TTT GTT ATG GCC AGG GAA CAT GCT CCA TCA ATT	768
Met Val Arg Glu Leu Phe Val Met Ala Arg Glu His Ala Pro Ser Ile	
245 250 255	
ATC TTC ATG GAT GAA ATT GAC AGT ATT GGA TCT GCT CGG ATG GAA TCT	816
Ile Phe Met Asp Glu Ile Asp Ser Ile Gly Ser Ala Arg Met Glu Ser	
260 265 270	
GGA AGT GGC AAC GGT GAT AGT GAG GTA CAG CGT ACT ATG CTG GAA CTT	864
Gly Ser Gly Asn Gly Asp Ser Glu Val Gln Arg Thr Met Leu Glu Leu	
275 280 285	
CTC AAC CAG TTG GAT GGA TTT GAA GCT TCA AAT AAG ATC AAG GTT TTG	912
Leu Asn Gln Leu Asp Gly Phe Glu Ala Ser Asn Lys Ile Lys Val Leu	
290 295 300	
ATG GCC ACC AAT CGG ATT GAT ATC CTG GAT CAA GCC CTC CTT AGA CCA	960
Met Ala Thr Asn Arg Ile Asp Ile Leu Asp Gln Ala Leu Leu Arg Pro	
305 310 315 320	
GGA CGG ATA GAC CGG AAA ATT GAA TTT CCA ACC CCT AAT GAA GAG TCT	1008
Gly Arg Ile Asp Arg Lys Ile Glu Phe Pro Thr Pro Asn Glu Glu Ser	
325 330 335	
CGG CTG GAT ATT TTG AAA ATC CAT TCT AGA AGA ATG AAT TTA ATG CGT	1056
Arg Leu Asp Ile Leu Lys Ile His Ser Arg Arg Met Asn Leu Met Arg	
340 345 350	
GGC ATT GAT TTG AAG AAG ATT GCC GAG AAG ATG AAT GGA GCA TCT GGT	1104
Gly Ile Asp Leu Lys Lys Ile Ala Glu Lys Met Asn Gly Ala Ser Gly	
355 360 365	
GCT GAA CTT AAG GCT GTT TGC ACT GAA GCT GGA ATG TTT GCT TTG AGG	1152
Ala Glu Leu Lys Ala Val Cys Thr Glu Ala Gly Met Phe Ala Leu Arg	
370 375 380	
GAG CGG AGG GTA CAC GTG ACT CAG GAG GAT TTT GAG ATG GCC GTG GCG	1200
Glu Arg Arg Val His Val Thr Gln Glu Asp Phe Glu Met Ala Val Ala	
385 390 395 400	
AAG GTG ATG AAA AAG GAG ACT GAA AAA AAC ATG TCA TTG CGG AAG TTG	1248
Lys Val Met Lys Lys Glu Thr Glu Lys Asn Met Ser Leu Arg Lys Leu	
405 410 415	
TGG AAG	1254
Trp Lys	

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 418 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Leu Val Gly Val Glu Leu Lys His Ala Ala Glu Gly Val Pro
 1 5 10 15
 Glu Ala Asn Cys Ser Ala Lys Pro Thr Lys Gln Gly Glu Gly Leu Arg
 20 25 30
 His Tyr Tyr Ser Leu Asn Ile His Glu His Gln Leu Leu Leu Arg Gln
 35 40 45
 Lys Thr His Asn Leu Asn Arg Leu Glu Ala Gln Arg Asn Asp Leu Asn
 50 55 60
 Ser Arg Val Arg Met Leu Arg Glu Glu Leu Gln Leu Leu Gln Glu Pro
 65 70 75 80
 Gly Ser Tyr Val Gly Glu Val Val Lys Val Met Gly Lys Asn Lys Val
 85 90 95
 Leu Val Lys Val His Pro Glu Gly Lys Tyr Val Val Asp Ile Asp Lys
 100 105 110
 Asn Ile Asp Ile Thr Lys Ile Thr Pro Ser Thr Arg Val Ala Leu Arg
 115 120 125
 Asn Asp Ser Tyr Val Leu His Leu Val Leu Pro Ser Lys Val Asp Pro
 130 135 140
 Leu Val Asn Leu Met Lys Val Glu Lys Val Pro Asp Ser Thr Tyr Asp
 145 150 155 160
 Met Ile Gly Gly Leu Asp Gln Gln Ile Lys Glu Ile Lys Glu Val Ile
 165 170 175
 Glu Leu Pro Ile Lys His Pro Glu Leu Phe Glu Ser Leu Gly Ile Ala
 180 185 190
 Gln Pro Lys Gly Val Leu Leu Tyr Gly Pro Pro Gly Thr Gly Lys Thr
 195 200 205
 Leu Leu Ala Arg Ala Val Ala His His Thr Asp Cys Thr Phe Ile Arg
 210 215 220
 Val Ser Gly Ser Glu Leu Val Gln Lys Tyr Ile Gly Glu Gly Ser Arg
 225 230 235 240
 Met Val Arg Glu Leu Phe Val Met Ala Arg Glu His Ala Pro Ser Ile
 245 250 255
 Ile Phe Met Asp Glu Ile Asp Ser Ile Gly Ser Ala Arg Met Glu Ser
 260 265 270
 Gly Ser Gly Asn Gly Asp Ser Glu Val Gln Arg Thr Met Leu Glu Leu
 275 280 285
 Leu Asn Gln Leu Asp Gly Phe Glu Ala Ser Asn Lys Ile Lys Val Leu
 290 295 300
 Met Ala Thr Asn Arg Ile Asp Ile Leu Asp Gln Ala Leu Leu Arg Pro
 305 310 315 320

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CTC ATG AAA GTT GAG AAG GTT CCG GAT TCT ACC TAT GAT ATG ATT GGA Leu Met Lys Val Glu Lys Val Pro Asp Ser Thr Tyr Asp Met Ile Gly 115 120 125	383
GGC CTT GAC CAG CAA ATT AAA GAG ATC AAA GAG GTC ATT GAG CTT CCA Gly Leu Asp Gln Gln Ile Lys Glu Ile Lys Glu Val Ile Glu Leu Pro 130 135 140	431
ATC AAA CAT CCG GAA CTG TTT GAG AGC CTT GGA ATT GCG CAA CCA AAG Ile Lys His Pro Glu Leu Phe Glu Ser Leu Gly Ile Ala Gln Pro Lys 145 150 155	479
GGT GTC CTT CTT TAT GGA CCT CCG GGC ACA GGA AAG ACA TTG TTG GCA Gly Val Leu Leu Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Leu Ala 160 165 170 175	527
CGT GCG GTT GCT CAT CAC ACT GAC TGC ACC TTC ATC AGG GTG TCT GGT Arg Ala Val Ala His His Thr Asp Cys Thr Phe Ile Arg Val Ser Gly 180 185 190	575
TCT GAG TTG GTT CAG AAG TAT ATT GGT GAG GGC TCC CGG ATG GTT AGG Ser Glu Leu Val Gln Lys Tyr Ile Gly Glu Gly Ser Arg Met Val Arg 195 200 205	623
GAA CTC TTT GTT ATG GCC AGG GAA CAT GCA CCA TCC ATT ATA TTT ATG Glu Leu Phe Val Met Ala Arg Glu His Ala Pro Ser Ile Ile Phe Met 210 215 220	671
GAT GAA ATT GAC TCT ATC GGA TCT GCT AGA ATG GAG TCT GGA ACT GGC Asp Glu Ile Asp Ser Ile Gly Ser Ala Arg Met Glu Ser Gly Thr Gly 225 230 235	719
AAC GGT GAC AGT GAA GTT CAG CGT ACT ATG CTT GAA CTT CTA AAC CAG Asn Gly Asp Ser Glu Val Gln Arg Thr Met Leu Glu Leu Leu Asn Gln 240 245 250 255	767
CTC GAT GGT TTT GAA GCA TCA AAC AAA ATT AAG GTT TTG ATG GCA ACG Leu Asp Gly Phe Glu Ala Ser Asn Lys Ile Lys Val Leu Met Ala Thr 260 265 270	815
AAC AGA ATA GAC ATT TTG GAT CAA GCC CTT CTG AGG CCT GGC CGC ATA Asn Arg Ile Asp Ile Leu Asp Gln Ala Leu Leu Arg Pro Gly Arg Ile 275 280 285	863
GAC AGG AAG ATT GAA TTT CCA AAT CCT AAC GAG GAT TCA CGT TTC GAT Asp Arg Lys Ile Glu Phe Pro Asn Pro Asn Glu Asp Ser Arg Phe Asp 290 295 300	911
ATC TTG AAG ATC CAT TCA AGA AAA ATG AAC TTG ATG CGT GGC ATT GAT Ile Leu Lys Ile His Ser Arg Lys Met Asn Leu Met Arg Gly Ile Asp 305 310 315	959
CTG AAA AAG ATC GCG GAA AAG ATG AAT GGG GCC TCA GGA GCT GAG CTC Leu Lys Lys Ile Ala Glu Lys Met Asn Gly Ala Ser Gly Ala Glu Leu 320 325 330 335	1007
AAG GCC GTC TGC ACA GAG GCT GGA ATG TTT GCT CTT CGT GAG AGA AGG Lys Ala Val Cys Thr Glu Ala Gly Met Phe Ala Leu Arg Glu Arg Arg 340 345 350	1055
GTG CAC GTT ACC CAG GAG GAC TTC GAG ATG GCA GTG GCC AAG GTG ATG Val His Val Thr Gln Glu Asp Phe Glu Met Ala Val Ala Lys Val Met 355 360 365	1103

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AAG AAA GAC ACG GAG AAG AAC ATG TCC CTG CGC AAG CTC TGG AAG 1148
 Lys Lys Asp Thr Glu Lys Asn Met Ser Leu Arg Lys Leu Trp Lys
 370 375 380

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 382 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu His Ile His Asp Leu Gln Leu Gln Ile Arg Gln Lys Thr His Asn
 1 5 10 15
 Leu Asn Arg Leu Glu Ala Gln Arg Asn Asp Leu Asn Ser Arg Val Arg
 20 25 30
 Met Leu Arg Glu Glu Leu Gln Leu Leu Gln Glu Pro Gly Ser Tyr Val
 35 40 45
 Gly Glu Val Val Lys Val Met Gly Lys Ser Lys Val Leu Val Lys Val
 50 55 60
 His Pro Glu Gly Lys Tyr Val Val Asp Ile Asp Lys Ser Ile Asp Ile
 65 70 75 80
 Thr Lys Ile Thr Pro Ser Thr Arg Val Ala Leu Arg Asn Asp Ser Tyr
 85 90 95
 Met Leu His Leu Ile Leu Pro Ser Lys Val Asp Pro Leu Val Asn Leu
 100 105 110
 Met Lys Val Glu Lys Val Pro Asp Ser Thr Tyr Asp Met Ile Gly Gly
 115 120 125
 Leu Asp Gln Gln Ile Lys Glu Ile Lys Glu Val Ile Glu Leu Pro Ile
 130 135 140
 Lys His Pro Glu Leu Phe Glu Ser Leu Gly Ile Ala Gln Pro Lys Gly
 145 150 155 160
 Val Leu Leu Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Leu Ala Arg
 165 170 175
 Ala Val Ala His His Thr Asp Cys Thr Phe Ile Arg Val Ser Gly Ser
 180 185 190
 Glu Leu Val Gln Lys Tyr Ile Gly Glu Gly Ser Arg Met Val Arg Glu
 195 200 205
 Leu Phe Val Met Ala Arg Glu His Ala Pro Ser Ile Ile Phe Met Asp
 210 215 220
 Glu Ile Asp Ser Ile Gly Ser Ala Arg Met Glu Ser Gly Thr Gly Asn
 225 230 235 240
 Gly Asp Ser Glu Val Gln Arg Thr Met Leu Glu Leu Leu Asn Gln Leu
 245 250 255
 Asp Gly Phe Glu Ala Ser Asn Lys Ile Lys Val Leu Met Ala Thr Asn
 260 265 270

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Arg Ile Asp Ile Leu Asp Gln Ala Leu Leu Arg Pro Gly Arg Ile Asp
 275 280 285

Arg Lys Ile Glu Phe Pro Asn Pro Asn Glu Asp Ser Arg Phe Asp Ile
 290 295 300

Leu Lys Ile His Ser Arg Lys Met Asn Leu Met Arg Gly Ile Asp Leu
 305 310 315 320

Lys Lys Ile Ala Glu Lys Met Asn Gly Ala Ser Gly Ala Glu Leu Lys
 325 330 335

Ala Val Cys Thr Glu Ala Gly Met Phe Ala Leu Arg Glu Arg Arg Val
 340 345 350

His Val Thr Gln Glu Asp Phe Glu Met Ala Val Ala Lys Val Met Lys
 355 360 365

Lys Asp Thr Glu Lys Asn Met Ser Leu Arg Lys Leu Trp Lys
 370 375 380

2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1358 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCAAGCCAA CAAGCAAGAC CGCTTCTGCT TGTGATCCGA AACCTTTCTT CGAACGCAAA 60

AAGAACCCCA CCCGACGTAC CGCGAGCCGG CGATGGCGAC GGTGGCGATG GACATCTCGA 120

AGCCCACGCC GGCAGCGTCC GGTGACGAGG CAGCAGCGGC GGCGAAGGGG AGGAGCGGCG 180

GCGGGGGCGA GGGGTGCGG CAGTACTACC TGCAGCACAT CCATGACCTG CAGCTCCAGA 240

TCCGGCAGAA GACCCATAAC CTCAACCGCC TCGAGGCCCA GCGCAACGAC CTCAACTCCC 300

GAGTTAGAAT GCTCAGGGAA GAGTTGCAGT TGCTTCAAGA GCCTGGCTCA TATGTTGGTG 360

AGGTGGTGAA GGTGATGGGG AAATCAAAGG TTCTGGTGAA GGTACATCCC GAAGGCAAAT 420

ATGTGGTGGA TATAGATAAG AGCATTGATA TCACTAAGAT CACACCTTCA ACAAGAGTTG 480

CTCTTCGGAA TGACAGCTAT ATGCTCCATC TGATCCTACC AAGCAAAGTT GATCCATTGG 540

TCAATCTCAT GAAAGTTGAG AAGGTTCCGG ATTCTACCTA TGATATGATT GGAGGCCTTG 600

ACCAGCAAAT TAAAGAGATC AAAGAGGTCA TTGAGCTTCC AATCAAACAT CCGGAAGTGT 660

TTGAGAGCCT TGGAATTGCG CAACCAAAGG GTGTCCTTCT TTATGGACCT CCGGGCACAG 720

GAAAGACATT GTTGGCACGT GCGGTTGCTC ATCACACTGA CTGCACCTTC ATCAGGGTGT 780

CTGGTTCTGA GTTGGTTCAG AAGTATATTG GTGAGGGCTC CCGGATGGTT AGGGAAGTCT 840

TTGTTATGGC CAGGGAACAT GCACCATCCA TTATATTTAT GGATGAAATT GACTCTATCG 900

GATCTGCTAG AATGGAGTCT GGAAGTGGCA ACGGTGACAG TGAAGTTCAG CGTACTATGC 960

TTGAACTTCT AAACCAGCTC GATGGTTTTG AAGCATCAAA CAAAATTAAG GTTTTGATGG 1020

Thr Phe Ile Arg Val Ser Gly Ser Glu Leu Val Gln Lys Tyr Ile Gly
 225 230 235 240

Glu Gly Ser Arg Met Val Arg Glu Leu Phe Val Met Ala Arg Glu His
 245 250 255

Ala Pro Ser Ile Ile Phe Met Asp Glu Ile Asp Ser Ile Gly Ser Ala
 260 265 270

Arg Met Glu Ser Gly Thr Gly Asn Gly Asp Ser Glu Val Gln Arg Thr
 275 280 285

Met Leu Glu Leu Leu Asn Gln Leu Asp Gly Phe Glu Ala Ser Asn Lys
 290 295 300

Ile Lys Val Leu Met Ala Thr Asn Arg Ile Asp Ile Leu Asp Gln Ala
 305 310 315 320

Leu Leu Arg Pro Gly Arg Ile Asp Arg Lys Ile Glu Phe Pro Asn Pro
 325 330 335

Asn Glu Asp Ser Arg Phe Asp Ile Leu Lys Ile His Ser Arg Lys Met
 340 345 350

Asn Leu Met Arg Gly Ile Asp Leu Lys Lys Ile Ala Glu Lys Met Asn
 355 360 365

Gly Ala Ser Gly Ala Glu Leu Lys Ala Val Cys Thr Glu Ala Gly Met
 370 375 380

Phe Ala Leu Arg Glu Arg Arg Val His Val Thr Gln Glu Asp Phe Glu
 385 390 395 400

Met Ala Val Ala Lys Val Met Lys Lys Asp Thr Glu Lys Asn Met Ser
 405 410 415

Leu Arg Lys Leu Trp Lys
 420

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 406 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ala Leu Asp Gly Pro Glu Gln Met Glu Leu Glu Glu Gly Lys Ala
 1 5 10 15

Gly Ser Gly Leu Arg Gln Tyr Tyr Leu Ser Lys Ile Glu Glu Leu Gln
 20 25 30

Leu Ile Val Asn Asp Lys Ser Gln Asn Leu Arg Arg Leu Gln Ala Gln
 35 40 45

Arg Asn Glu Leu Asn Ala Lys Val Arg Leu Leu Arg Glu Glu Leu Gln
 50 55 60

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Leu Leu Gln Glu Gln Gly Ser Tyr Val Gly Glu Val Val Arg Ala Met
 65 70 75 80
 Asp Lys Lys Lys Val Leu Val Lys Val His Pro Glu Gly Lys Phe Val
 85 90 95
 Val Asp Val Asp Lys Asn Ile Asp Ile Asn Asp Val Thr Pro Asn Cys
 100 105 110
 Arg Val Ala Leu Arg Asn Asp Ser Tyr Thr Leu His Lys Ile Leu Pro
 115 120 125
 Asn Lys Val Asp Pro Leu Val Ser Leu Met Met Val Glu Lys Val Pro
 130 135 140
 Asp Ser Thr Tyr Glu Met Ile Gly Gly Leu Asp Lys Gln Ile Lys Glu
 145 150 155 160
 Ile Lys Glu Val Ile Glu Leu Pro Val Lys His Pro Glu Leu Phe Glu
 165 170 175
 Ala Leu Gly Ile Ala Gln Pro Lys Gly Val Leu Leu Tyr Gly Pro Pro
 180 185 190
 Gly Thr Gly Lys Thr Leu Leu Ala Arg Ala Val Ala His His Thr Asp
 195 200 205
 Cys Thr Phe Ile Arg Val Ser Gly Ser Glu Leu Val Gln Lys Phe Ile
 210 215 220
 Gly Glu Gly Ala Arg Met Val Arg Glu Leu Phe Val Met Ala Arg Glu
 225 230 235 240
 His Ala Pro Ser Ile Ile Phe Met Asp Glu Ile Asp Ser Ile Gly Ser
 245 250 255
 Ser Arg Leu Glu Gly Gly Ser Gly Gly Asp Ser Glu Val Gln Arg Thr
 260 265 270
 Met Leu Glu Leu Leu Asn Gln Leu Asp Gly Phe Glu Ala Thr Lys Asn
 275 280 285
 Ile Lys Val Ile Met Ala Thr Asn Arg Ile Asp Ile Leu Asp Ser Ala
 290 295 300
 Leu Leu Arg Pro Gly Arg Ile Asp Arg Lys Ile Glu Phe Pro Pro Pro
 305 310 315 320
 Asn Glu Glu Ala Arg Leu Asp Ile Leu Lys Ile His Ser Arg Lys Met
 325 330 335
 Asn Leu Thr Arg Gly Ile Asn Leu Arg Lys Ile Ala Glu Leu Met Pro
 340 345 350
 Gly Ala Ser Gly Ala Glu Val Lys Gly Val Cys Thr Glu Ala Gly Met
 355 360 365
 Tyr Ala Leu Arg Glu Arg Arg Val His Val Thr Gln Glu Asp Phe Glu
 370 375 380
 Met Ala Val Ala Lys Val Met Gln Lys Asp Ser Glu Lys Asn Met Ser
 385 390 395 400
 Ile Lys Lys Leu Trp Lys
 405

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 405 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Yeast

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Met Thr Ala Ala Val Thr Ser Ser Asn Ile Val Leu Glu Thr His Glu
  1          5          10          15
Ser Gly Ile Lys Pro Tyr Phe Glu Gln Lys Ile Gln Glu Thr Glu Leu
  20          25          30
Lys Ile Arg Ser Lys Thr Glu Asn Val Arg Arg Leu Glu Ala Gln Arg
  35          40          45
Asn Ala Leu Asn Asp Lys Val Arg Phe Ile Lys Asp Glu Leu Arg Leu
  50          55          60
Leu Gln Glu Pro Gly Ser Tyr Val Gly Glu Val Ile Lys Ile Val Ser
  65          70          75          80
Asp Lys Lys Val Leu Val Lys Val Gln Pro Glu Gly Lys Tyr Ile Val
  85          90          95
Asp Val Ala Lys Asp Ile Asn Val Lys Asp Leu Lys Ala Ser Gln Arg
 100          105          110
Val Cys Leu Arg Ser Asp Ser Tyr Met Leu His Lys Val Leu Glu Asn
 115          120          125
Lys Ala Asp Pro Leu Val Ser Leu Met Met Val Glu Lys Val Pro Asp
 130          135          140
Ser Thr Tyr Asp Met Val Gly Gly Leu Thr Lys Gln Ile Lys Glu Ile
 145          150          155          160
Lys Glu Val Ile Glu Leu Pro Val Lys His Pro Glu Leu Phe Glu Ser
 165          170          175
Leu Gly Ile Ala Gln Pro Lys Gly Val Ile Leu Tyr Gly Pro Pro Gly
 180          185          190
Thr Gly Lys Thr Leu Leu Ala Arg Ala Val Ala His His Thr Asp Cys
 195          200          205
Lys Phe Ile Arg Val Ser Gly Ala Glu Leu Val Gln Lys Tyr Ile Gly
 210          215          220
Glu Gly Ser Arg Met Val Arg Glu Leu Phe Val Met Ala Arg Glu His
 225          230          235          240
Ala Pro Ser Ile Ile Phe Met Asp Glu Ile Asp Ser Ile Gly Ser Thr
 245          250          255
Arg Val Glu Gly Ser Gly Gly Gly Asp Ser Glu Val Gln Arg Thr Met
 260          265          270

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Leu Glu Leu Leu Asn Gln Leu Asp Gly Phe Glu Thr Ser Lys Asn Ile
 275 280 285
 Lys Ile Ile Met Ala Thr Asn Arg Leu Asp Ile Leu Asp Pro Ala Leu
 290 295 300
 Leu Arg Pro Gly Arg Ile Asp Arg Lys Ile Glu Phe Pro Pro Pro Ser
 305 310 315 320
 Val Ala Ala Arg Ala Glu Ile Leu Arg Ile His Ser Arg Lys Met Asn
 325 330 335
 Leu Thr Arg Gly Ile Asn Leu Arg Lys Val Ala Glu Lys Met Asn Gly
 340 345 350
 Cys Ser Gly Ala Asp Val Lys Gly Val Cys Thr Glu Ala Gly Met Tyr
 355 360 365
 Ala Leu Arg Glu Arg Arg Ile His Val Thr Gln Glu Asp Phe Glu Leu
 370 375 380
 Ala Val Gly Lys Val Met Asn Lys Asn Gln Glu Thr Ala Ile Ser Val
 385 390 395 400
 Ala Lys Leu Phe Lys
 405

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 406 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Human Tripl

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ala Leu Asp Gly Pro Glu Gln Met Glu Leu Glu Glu Gly Lys Ala
 1 5 10 15
 Gly Ser Gly Leu Arg Gln Tyr Tyr Leu Ser Lys Ile Glu Glu Leu Gln
 20 25 30
 Leu Ile Val Asn Asp Lys Ser Gln Asn Leu Arg Arg Leu Gln Ala Gln
 35 40 45
 Arg Asn Glu Leu Asn Ala Lys Val Arg Leu Leu Arg Glu Glu Leu Gln
 50 55 60
 Leu Leu Gln Glu Gln Gly Ser Tyr Val Gly Glu Val Val Arg Ala Met
 65 70 75 80
 Asp Lys Lys Lys Val Leu Val Lys Val His Pro Glu Gly Lys Phe Val
 85 90 95
 Val Asp Val Asp Lys Asn Ile Asp Ile Asn Asp Val Thr Pro Asn Cys
 100 105 110
 Arg Val Ala Leu Arg Asn Asp Ser Tyr Thr Leu His Lys Ile Leu Pro
 115 120 125

Asn Lys Val Asp Pro Leu Val Ser Leu Met Met Val Glu Lys Val Pro
 130 135 140
 Asp Ser Thr Tyr Glu Met Ile Gly Gly Leu Asp Lys Gln Ile Lys Glu
 145 150 155 160
 Ile Lys Glu Val Ile Glu Leu Pro Val Lys His Pro Glu Leu Phe Glu
 165 170 175
 Ala Leu Gly Ile Ala Gln Pro Lys Gly Val Leu Leu Tyr Gly Pro Pro
 180 185 190
 Gly Thr Gly Lys Thr Leu Leu Ala Arg Ala Val Ala His His Thr Asp
 195 200 205
 Cys Thr Phe Ile Arg Val Ser Gly Ser Glu Leu Val Gln Lys Phe Ile
 210 215 220
 Gly Glu Gly Ala Arg Met Val Arg Glu Leu Phe Val Met Ala Arg Glu
 225 230 235 240
 His Ala Pro Ser Ile Ile Phe Met Asp Glu Ile Asp Ser Ile Gly Ser
 245 250 255
 Ser Arg Leu Glu Gly Gly Ser Gly Gly Ser Ser Glu Val Gln Arg Gln
 260 265 270
 Met Leu Glu Leu Leu Asn Gln Leu Asp Gly Phe Glu Ala Thr Lys Asn
 275 280 285
 Ile Lys Val Ile Met Ala Thr Asn Arg Ile Asp Met Leu Asp Ser Ala
 290 295 300
 Leu Leu Arg Pro Gly Arg Ile Asp Arg Lys Ile Glu Phe Pro Pro Pro
 305 310 315 320
 Asn Glu Glu Ala Arg Leu Asp Ile Leu Lys Ile His Ser Arg Lys Met
 325 330 335
 Asn Leu Thr Arg Gly Ile Asn Leu Arg Lys Ile Ala Glu Leu Met Pro
 340 345 350
 Gly Ala Ser Gly Ala Glu Val Lys Gly Val Cys Thr Glu Ala Gly Met
 355 360 365
 Tyr Ala Leu Arg Glu Arg Arg Val His Val Thr Gln Glu Asp Phe Glu
 370 375 380
 Met Ala Val Ala Lys Val Met Gln Lys Asp Ser Glu Lys Asn Met Ser
 385 390 395 400
 Ile Lys Lys Leu Trp Lys
 405

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 406 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Human p45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Leu Asp Gly Pro Glu Gln Met Glu Leu Glu Glu Gly Lys Ala
 1 5 10 15

Gly Ser Gly Leu Arg Gln Tyr Tyr Leu Ser Lys Ile Glu Glu Leu Gln
 20 25 30

Leu Ile Val Asn Asp Lys Ser Gln Asn Leu Arg Arg Leu Gln Ala Gln
 35 40 45

Arg Asn Glu Leu Asn Ala Lys Val Arg Leu Leu Arg Arg Glu Leu Gln
 50 55 60

Leu Leu Gln Glu Gln Gly Ser Tyr Val Gly Glu Val Val Arg Ala Met
 65 70 75 80

Asp Lys Lys Lys Val Leu Val Lys Val His Pro Glu Gly Lys Phe Val
 85 90 95

Val Asp Val Asp Lys Asn Ile Asp Ile Asn Asp Val Thr Pro Asn Cys
 100 105 110

Arg Val Ala Leu Arg Asn Asp Ser Tyr Thr Leu His Lys Ile Leu Pro
 115 120 125

Asn Lys Val Asp Pro Leu Val Ser Leu Met Met Val Glu Lys Val Pro
 130 135 140

Asp Ser Thr Tyr Glu Met Ile Gly Gly Leu Asp Lys Gln Ile Lys Glu
 145 150 155 160

Ile Lys Glu Val Ile Glu Leu Pro Val Lys His Pro Glu Leu Phe Glu
 165 170 175

Ala Leu Gly Ile Ala Gln Pro Lys Gly Val Leu Leu Tyr Gly Pro Pro
 180 185 190

Gly Thr Gly Lys Thr Leu Leu Ala Arg Ala Val Ala His His Thr Asp
 195 200 205

Cys Thr Phe Ile Arg Val Ser Gly Ser Glu Leu Val Gln Lys Phe Ile
 210 215 220

Gly Glu Gly Ala Arg Met Val Arg Glu Leu Phe Val Met Ala Arg Glu
 225 230 235 240

His Ala Pro Ser Ile Ile Phe Met Asp Glu Ile Asp Ser Ile Gly Ser
 245 250 255

Ser Arg Leu Glu Gly Gly Ser Gly Gly Asp Ser Glu Val Gln Arg Thr
 260 265 270

Met Leu Glu Leu Leu Asn Gln Leu Asp Gly Phe Glu Ala Thr Lys Asn
 275 280 285

Ile Lys Val Ile Met Ala Thr Asn Arg Ile Asp Ile Leu Asp Ser Ala
 290 295 300

Leu Leu Arg Pro Gly Arg Ile Asp Arg Lys Ile Glu Phe Pro Pro Pro
 305 310 315 320

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Asn Glu Glu Ala Arg Leu Asp Ile Leu Lys Ile His Ser Arg Lys Met
 325 330 335

Asn Leu Thr Arg Gly Ile Asn Leu Arg Lys Ile Ala Glu Leu Met Pro
 340 345 350

Gly Ala Ser Gly Ala Glu Val Lys Gly Val Cys Thr Glu Ala Gly Met
 355 360 365

Tyr Ala Leu Arg Glu Arg Arg Val His Val Thr Gln Glu Asp Phe Glu
 370 375 380

Met Ala Val Ala Lys Val Met Gln Lys Asp Ser Glu Lys Asn Met Ser
 385 390 395 400

Ile Lys Lys Leu Trp Lys
 405

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 548 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: wlln.pk0053.g3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCCGACCGA CCTACCCTAA TCGGCGATGG CGACGGTGGC GATGGACATC TCGAAGCCCA	60
CTCCAGTGGC GTCCGGCGAC GAGGCCGCGG CGGCGGCCAA GGGGAGGAGC GGCGGAGGGG	120
GCGAGGGGCT GCGGCAGTAC TACCTGCAGC ACATCCACGA CCTGCAGCTC CAGATCCGGC	180
ATAAGACCCA CAACCTCAAC CGTCTCGAGG CCCAGCGCAA TGACCTCAAC TCCCGAGTTA	240
GAATGCTCAG GGAAGATTGC ANTTGCTTCA NGAGCCTGGC TCATATGTTG GTNAGGTGGT	300
GAAGGCATGG GGAAATCAAA GGTTCTGGGT NAAGGTAAAC CCCGAAGGCA AATANNTTGT	360
TGATATAAAT AANCCATTGA TTCNCANGTT NACACCTNCA ACAANANCGC CTCGAANTG	420
ATACNANNGC CCATCTGTTT TACCACCAAA TTNNCATTGG CAACCCCATG AANTTAAAAG	480
TCCTGATCAC ATAAAANCTT TGGGGCCTGC CACCCTTNAG AAATANNGTT CTGTGTTCAA	540
NAAAACGG	548

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 115 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: wlln.pk0053.g3

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ala Thr Val Ala Met Asp Ile Ser Lys Pro Thr Pro Val Ala Ser
 1 5 10 15
 Gly Asp Glu Ala Ala Ala Ala Lys Gly Arg Ser Gly Gly Gly Gly
 20 25 30
 Glu Gly Leu Arg Gln Tyr Tyr Leu Gln His Ile His Asp Leu Gln Leu
 35 40 45
 Gln Ile Arg His Lys Thr His Asn Leu Asn Arg Leu Glu Ala Gln Arg
 50 55 60
 Asn Asp Leu Asn Ser Arg Val Arg Met Leu Arg Glu Asp Xaa Xaa Leu
 65 70 75 80
 Leu Xaa Glu Pro Gly Ser Tyr Val Gly Xaa Val Val Lys Ala Trp Gly
 85 90 95
 Asn Gln Arg Phe Trp Val Lys Val Asn Pro Glu Gly Lys Xaa Xaa Val
 100 105 110
 Asp Ile Asn
 115

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 593 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: rlr6.pk0064.e10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTTTAAACGG TACAGGAAAA ACGCTACTAG CTCGGGCAGT TGCTCATCAC ACCGACTGTA 60
 CCTTTATTAA GGGTGTGAGG TTCCGAGTTG GTTCAGAAGT ATATTGGAGA GGGTTCCAGA 120
 ATGGTTCGTG AACTATTTGT GATGGCCANA GAGCATGCAC CATCCATAAT CTTTATGGAT 180
 GAAATAGACT CCATTGGATC TGCTANAATG CAGTCANGAT CTGGGGGTGG TGATAGTGAG 240
 GTTCAACGCA CTATGCTTGA TCTTCTGAAT CAACTTGATG GCTTTGAAGC ATCAAACANA 300
 ATTAAGGTCT TATGGCGACA AATANGATGG ATATTTTGGA TCAAGCTCTC TGANGCCTGG 360
 TCGCATTGAT AGGAAGATGA ATTTCCAATC CGAATGAAGA TCCGCTTGAT ANTTGAAGAT 420
 CATTCAAGAA AAATGACTGA TCTGGATTGT CTGAAAAGAT GCAGAGAAAT GATGGGCNCT 480
 GGACAAACTA AGNGTCTNAC GAACAAGATT TGCCTCCAAA NAGGTATTCA CAGAGATCTA 540
 TGGGTGCAAG GTGAAAGNAC GAAGAATCCG NCACCGAATA AGANNCACCA ACC 593

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 126 amino acids
 (B) TYPE: amino acid

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(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: rlr6.pk0064.e10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly	Thr	Gly	Lys	Thr	Leu	Leu	Ala	Arg	Ala	Val	Ala	His	His	Thr	Asp
1				5					10					15	
Cys	Thr	Phe	Xaa	Arg	Val	Ser	Gly	Ser	Glu	Leu	Val	Gln	Lys	Tyr	Ile
			20					25					30		
Gly	Glu	Gly	Ser	Arg	Met	Val	Arg	Glu	Leu	Phe	Val	Met	Ala	Xaa	Glu
		35					40					45			
His	Ala	Pro	Ser	Ile	Ile	Phe	Met	Asp	Glu	Ile	Asp	Ser	Ile	Gly	Ser
	50					55					60				
Ala	Xaa	Met	Gln	Ser	Xaa	Ser	Gly	Gly	Gly	Asp	Ser	Glu	Val	Gln	Arg
65					70					75					80
Thr	Met	Leu	Asp	Leu	Leu	Asn	Gln	Leu	Asp	Gly	Phe	Glu	Ala	Ser	Asn
				85					90						95
Xaa	Ile	Lys	Val	Xaa	Met	Ala	Thr	Asn	Xaa	Met	Asp	Ile	Leu	Asp	Gln
			100					105					110		
Ala	Leu	Xaa	Xaa	Pro	Gly	Arg	Ile	Asp	Arg	Lys	Met	Asn	Phe		
		115					120					125			